Remarks

This application is a Continuation application under 37 CFR §1.53 (b).

Claims 1-20 have been cancelled and replaced with Claims 21-26. Support for claims 21-26 can be found in the specification on page 34, last two lines through line 3 on page 36 and in the Examples. Thus, no new matter has been added.

The present application is a Continuation application of Application No. 09/049,304 filed March 27, 1998 which is a continuation-in-part of Application No. 08/824,627 filed March 27, 1997 which is a continuation-in-part of Application No. 08/474,633 filed June 7, 1995 which is a continuation-in-part of Application No. 08/178,212 filed January 6, 1994 (now abandoned) which is a §371 of PCT/US93/02480 filed March 18, 1993 which is a continuation-in-part of Application No. 07/855,414 filed March 19, 1992 (now abandoned).

Submitted herewith in Appendix A is a comparison of the claimed amino acid sequence (SEQ ID NO:122), encoded by SEQ ID NO:120, with the bifunctional *Arabidopsis* LKR-SDH protein (SEQ ID NO:111). This comparison demonstrates that the sequence of the invention has about 60% homology with the published *Arabidopsis* sequence (SEQ ID NO:111).

Attention is kindly invited to Tang et al. (*Plant Cell* 9:1305-1316 (1997), copy enclosed) and Epelbaum et al. (*Plant Mol. Biol.* 35:735-748 (1997), copy enclosed), which disclosed the *Arabidopsis* LKR-SDH sequence. Bifunctional and monofunctional versions of the LKR-SDH protein have been identified in mammals and plants.

The aforementioned publications discuss the LKR and SDH domains of the bifunctional protein that were identified by homology to the corresponding monofunctional proteins from yeast, showing 25% and 37% identity, respectively and by expressing the LKR and SDH domains of the bifunctional LKR-SDH separately in bacteria or yeast. The expression studies showed that the separate LKR and SDH domains conferred the expected activity and specifictiy to the transformed cells. The LKR and SDH domains have been boxed in Appendix A to faciliate review of the enclosed Appendix A. It should also be noted that, in addition to the LKR and SDH domains, a high degree of homology is also observed in the intermediary or 'spacer' region of the bifunctional LKR-SDH polypeptide.

As has been described in Dr. Carl Falco's Declaration, dated August 24th, 2000, (copy enclosed), a part of the corn LKR-SDH sequence (SEQ ID NO:122) was successfully used in cosupression studies and cosupression constructs to produce seeds having an increased accumulation of lysine. This increase in lysine appeared to be directly related to the cosupression of LKR-SDH.

It is respectfully submitted that the information presented in this preliminary amendment does indeed make a correlation between the teachings of Tang et al., Dr. Falco's Declaration and the claimed sequences.

A Petition for a three (3) month Extension of time was filed with papers for the Continuation Application on Friday, March 19, 2004. Thus, it is believed that no further Extension of Time is needed. However, if this belief is in error, then please charge any fees or credit any overpayment which are associated with the filing of this Preliminary Amendment to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

It is respectfully submitted that the claims are now in form for allowance which allowance is respectfully requested.

Respectfully submitted,

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APPENDIX A

Appendix A shows a comparison of the amino acid sequences of the bifunctional LKR-SDH proteins from *Arabidospis* and corn, SEQ ID NO:112 and 122, respectively. Amino acids conserved among both sequences are indicated with an asterisk (*) on the top row; dashes are used by the program to maximize alignment of the sequences. The LKR and SDH domains (boxed sequences) were identified by Epelbaum et al. (*Plant Mol. Biol.* 35:735-748 (1997)) and Tang et al. (*Plant Cell* 9:1305-1316 (1997)).

```
                                                  ***** ** *      ** ****
SEQ ID: 112   MNSNGHEEEKKLGNGVVGILSETVNKWERRTPLTPSHCARLLHGG-KDRTGISRIVVQPS
SEQ ID: 122   ------------------------------------CARLLLGGGKNGPRVNRIIVQPS

                ****** **  ***** *** **** ***** * ***  ******************
SEQ ID:112    AKRIHHDALYEHVGCEISDDLSDCGLILGIKQPELEMILPERAYAFFSHTHKAQKENMPL
SEQ ID:122    TRRIHHDAQYEDAGCEISEDLSECGLIIGIKQPKLQMILSDRAYAFFSHTHKAQKENMPL

               ********* * ******** *** ***** ****** **********************
SEQ_ID_112    LDKILSERVTLCDYELIVGDHGKRLLAFGKYAGRAGLVDFLHGLGQRYLSLGYSTPFLSL
SEQ_ID_122    LDKILEERVSLFDYELIVGDDGKRSLAFGKFAGRAGLIDFLHGLGQRYLSLGYSTPFLSL

                                  LKR domain
               * * ** ********** * ****  *** **** ****** **** **************
SEQ_ID_112    GASYMYSSLAAAKAAVISVGEEIASQGLPLGICPLVFVFTGTGNVSLAQEIFKLLPHTF
SEQ_ID_122    GQSHMYPSLAAAKAAVIVVAEEIATFGLPSGICPIVFVFTGVGNVSQGAQEIFKLLPHTF

               *  **** *      *    ***** * ***  ** * * ****   *** *********
SEQ_ID_112    VEPSKLPELFVKDKGISQNGISTKRVYQVYGCIITSQDMVEHKDPSKSFDKADYYAHPEH
SEQ_ID_122    VDAEKLPEIF-QARNLSKQSQSTKRVFQLYGCVVTSRDIVSHKDPTRQFDKGDYYAHPEH

               * ***** * ** ** *********** **   *** *   * **** ************
SEQ_ID_112    YNPVFHEKISPYTSVLVNCMYWEKRFPCLLSTKQLQDLTKKGLPLVGICDITCDIGGSIE
SEQ_ID_122    YTPVFHERIAPYASVIVNCMYWEKRFPPLLNMDQLQQLMETGCPLVGVCDITCDIGGSIE

               * *  * *  **** ****** *** * ** * ********* ******** ***  *
SEQ_ID_112    FVNRATLIDSPFFRFNPSNNSYYDDMDGDGVLCMAVDILPTEFAKEASQHFGDILSGFVG
SEQ_ID_122    FINKSTSIERPFFRYDPSKNSYHDDMEGAGVVCLAVDILPTEFSKEASQHFGNILSRLVA

               ****      **  ******  * ** *********      *
SEQ_ID_112    SLASMTEISDLPAHLKRACISYRGELTSLYEYIPRMRKSNPEEAQDNIIANGVSSQRTFN
SEQ_ID_122    SLASVKQPAELPSYLRRACIAHAGRLTPLYEYIPRMRNTMIDLAPAKTNPL--PDKKYS

                ****************** ** ******* ** *** *  **********   **
SEQ_ID_112    ILVSLSGHLFDKFLINEALDMIEAAGGSFHLAKCELGQSADAESYSELEVGADDKRVLDQ
SEQ_ID_122    TLVSLSGHLFDKFLINEALDIIETAGGSFHLVRCEVGQSTDDMSYSELEVGADDTATLDK

               ****** ***          *     ******   *         *    *********
SEQ_ID_112    IIDSLTRLANPNEDYISPHREANKISLKIGKVQQ-ENEIKEKPEMTKKSGVLILGAGRVC
SEQ_ID_122    IIDSLTSLANEHGGDHDAGQEIE-LALKIGKVNEYETDVTIDKGGPK---ILILGAGRVC

               **** ****   *          * *       ******** *** *** **    *  *
SEQ_ID_112    RPAADFLASVRTISSQQWYKTYFGADSEEKTDVHVIVASLYLKDAKETVEGISDVEAVRL
SEQ_ID_122    RPAAEFLASYPDICT-------YGVDDHDADQIHVIVASLYQKDAEETVDGIENTTATQL
```

APPENDIX A (Continued)

SDH domain

```
                 ** *  **   **** ** ****** **  *  ******* *******   ***   **
SEQ_ID_112       DVSDSESLLKYVSQVDVVLSLLPASCHAVVAKTCIELKKHLVTASYVDDETSMLHEKAKS
SEQ_ID_122       DVADIGSLSDLVSQVEVVISLLPASFHAAIAGVCIELKKHMVTASYVDESMSNLSQAAKD

                 ** *** *********  * ****  **  *** * ************************
SEQ_ID_112       AGITILGEMGLDPGIDHMMAMKMINDAHIKKGKVKSFTSYCGGLPSPAAANNPLAYKFSW
SEQ_ID_122       AGVTILCEMGLDPGIDHLMSMKMIDEAHARKGKIKAFTSYCGGLPSPAAANNPLAYKFSW

                 ***** * * *** **  *  ***** *** ** * *   *******  *** ** **
SEQ_ID_112       NPAGAIRAGQNPAKYKSNGDIIHVDGKNLYDSAARFRVPNLPAFALECFPNRDSLVYGEH
SEQ_ID_122       NPAGALRSGKNPAVYKFLGETIHVDGHNLYESAKRLRLRELPAFALEHLPNRNSLIYGDL

                 ***  ** ** * * ****** ** **** ****      *    * *    *   **
SEQ_ID_112       YGIESEATTIFRGTLRYEGFSMIMATLSKLGFFDSEANQVLSTGKRITFGALLSNILNK-
SEQ_ID_122       YGISKEASTIYRATYRYEGFSEIMVTLSKTGFFDAANHPLLQDTSRPTYKGFLDELLNNI

                      *   *   *       *  ***   **** * *** *** **   *  * *
SEQ_ID_112       ---DADNESEPLAG-EEEISKRIIKLG--HSKETAAKAAKTIVFLGFNEEREVPSLCKSV
SEQ_ID_122       STINTDLDIEASGGYDDDLIARLLKLGCCKNKEIAVKTVKTIKFLGLHEETQIPKGCSSP

                 **  *  **   **  ***************       *** *******   **  ****
SEQ_ID_112       FDATCYLMEEKLAYSGNEQDMVLLHHEVEVEFLESKRIEKHTATLLEFGDIKNGQTTTAM
SEQ_ID_122       FDVICQRMEQRMAYGHNEQDMVLLHHEVEVEYPDGQPAEKHQATLLEFGKVENGRSTTAM

                 * *********** *   *  * ** ***  * * *** **   **** *  *
SEQ_ID_112       AKTVGIPAAIGALVLIEDKIKTRGVLRPLEAEVYLPALDILQAYGIKLMEKAE
SEQ_ID_122       ALTVGIPAAIGALLLLKNKVQTKGVIRPLQPEIYVPALEILESSGIKLVEKVF
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